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[Claim(s)]

[Claim 1] Soybean KUNITTSU (Kunitz) Carcinostatic operation reinforcement agent characterized by making type trypsin inhibitor or its derivative into an active ingredient.

[Detailed Description of the Invention]

[0001]

[Industrial Application] This invention relates to a carcinostatic operation reinforcement agent.

[0002]

[Description of the Prior Art] The protease inhibitor of vegetable origin is variously reported from the former. There is specificity in these inhibitor and [for example, corn trypsin inhibitor or Japanese pumpkin trypsin inhibitor] suppression activity is shown in trypsin and an active Hageman factor (Thromb.Res. 20, 149, 1980 grades) It is known that KUNITTSU (Kunitz) type trypsin inhibitor has an inhibitory effect in trypsin, plasma kallikrein, an active X factor, etc. among soybean typsin inhibitor. Moreover, although the activity of the bradykinin is discovered and a pain and blood vessel permeability increase in the case of inflammatory-edema sthenia, the scheme in which a Hageman factor and Kallikrein participate is proposed by the activity manifestation of the bradykinin.

[0003] However, the present condition is remaining in the stage of research by in vitro, and the relation between protease inhibitor and inflammation is in vivo. There are very few examples of a report the effect was proved [examples].

[0004] On the other hand, the ascites stores in patients, such as stomach cancer, a renal cancer, an ovarian cancer, and carcinoma of liver, and, in the case of the patient of lung cancer, pleural effusion often stores, and a tumor metastasizes into an abdominal cavity or a thoracic cavity by high probability by the postoperative dissemination of these solids tumor,

and the same stagnation as the result is accepted. Since the fall of plasma protein will be caused and a physical strength fall and other exacerbation of a patient will be caused if stagnation of the ascites arises, the fall of a curative effect and the necessity for abundant ****** arise, if stagnation of pleural effusion arises, respiratory failure will be brought about again, as a result the remedially serious end of cancer is brought about, such as speeding up introduction of a patient's quality of the plague.

[0005]

[Problem to be solved by the invention] the result in which this invention person examined many things about the relation of inflammation suppression and protease inhibitor -- soybean KUNITTSU (Kunitz) type trypsin inhibitor -- dividing -- the derivative -- in vivo It found out that it set and a remarkable effect was in suppression of the inflammatory edema. Moreover, thing the organization of the blood-vessel-permeability increase in inflammation and the blood-vessel-permeability increase in a solid tumor is [thing] similar (exsorption of the blood-vessel-permeability sthenia in a solid tumor and an inflammation part and a plasma component is similar) while paying one's attention and examining many things This inhibitor and its derivative have an effect also in a cancerous breast and ascites stagnation suppression, Namely, this inhibitor checks the blood vessel permeability in a cancer organization strongly, and in the system of an ascites tumor (Meth A and Sarcoma 180) [with the repetitive administration of the above-mentioned inhibitor] Knowledge that stagnation of the ascites is suppressed notably, it finds out bringing a survival advantage to a cancer-bearing mouse etc. and it becomes a medicine useful also on cancer treatment is acquired, and this invention is completed.

[0006]

[Means for solving problem] That is, in this invention, one is soybean KUNITTSU (Kunitz). It is the cancerous breast, ascites stagnation restrainer, and inflammatory-edema sthenia restrainer which make an active ingredient type trypsin inhibitor or its derivative. Other one is soybean KUNITTSU. (Kunitz) It is the carcinostatic operation reinforcement agent which makes an active ingredient type trypsin inhibitor or its derivative.

[0007] Soybean trypsin inhibitor is molecular weight about 20,000 KUNITTSU. (Kunitz) Borman - bark of type trypsin inhibitor and a molecular weight about 6000-8000 (Bowman-Birk) It is divided roughly into type trypsin inhibitor two times. Those fundamental character Methods in Enzymology 19 and 853 (1970), and "the new viewpoint (centering on chemical research) of tongue substantia-alba research" -- the 1738th page (1982) (KYORITSU SHUPPAN) etc. -- [it is clarified and] the -- inner -- the mechanism of typical KUNITTSU (Kunitz) inhibitor is already determined Trypsin inhibitor and Borman - bark (Bowman-Birk) (Eur.J.Biochem. and 32,417 (1973) --) J. That Biochem.74, 697 (1973), therefore they are chemical and biological methods (a cell culture, gene recombination, etc.) Today is possible also for the synthesis to depend.

[0008] ** of this invention is soybean KUNITTSU. (Kunitz) Type trypsin inhibitor (it is called Following KTI) although it is considered as an active ingredient KTI carrying out limited decomposition of the thing of soybean origin, and this -- in addition -- KTI the thing holding peculiar activity, and these -- the above -- chemical and the thing obtained by having compounded with biological methods are included.

[0009] KTI of soybean origin The concrete procedure of some [procedures / preparation] already is proposed. Although which procedure may be adopted, generally it is a soybean, Defatted soybean, soybean whey etc. is used as materials. These to an aqueous medium or polar organic solvents (for example, ethanol, acetone, etc.) Extraction to depend, Membrane separation, rough refining things are obtained by concentration by isoelectric-point sedimentation, the salting out, etc., and the fraction. The specimen further refined in this by gel filtration, the ion exchange, the physical variance, or the chemical adsorption means can be obtained. Procedure, for example, casein digestion [of KUNITTSU (Kunitz)] [, that the activity and purity are also well-known [the notation in this Description] It measures by this procedure and is Sigma. Shrine trypsin "Type XI" (7500 - 9000 BAEEUnit/mg activity protein) One mg] which set quantity to check to 1 Unit SDS It can measure with content polyacrylamide gel electrophoresis etc.

[0010] Although the quantity as an active ingredient is somewhat different with the degree of refining, a medication method, and residual activity 1.5 - KTI which has 2.5 Unit/mg activity It converts into protein weight and, in intraperitoneal injection, the range of 1-3000mg / 60kg weight and the content which appointed the range of /60kg weight of 1-300mg as a standard in the case of the intravenous injection are suitable in general.

[0011] A derivative is KTI which is protein. An antigenicity is reduced and it is the quality of a decomposition product (SH-protease etc.). It can be used for 1 or 2 or more purposes of increasing amphiphilicity, by granting the receiving increase (improvement of half-life in the living body) of stability, or hydrophobicity. Such a derivative is KTI. Various modification objects (SMA), for example, styrene maleic-acid anhydrate copolymer, and the partial esterification material, Although a connective with the thing of illustration etc., other connectives with a low-molecular modification object, etc. are mentioned [a polyethylene gley call, the vinyl ether copolymer, Piran, and West German public presentation patent / No. 315541] and the joint procedure with these belongs to known If the above 1 or two or more purposes are attained, it is good by any procedures. For example, a modification object is SMA. when [or] it is the partial esterification material KTI A direct reaction is carried out in neutrality or an alkaline solution to N-end amino group or epsilon - amino group of lysine residue. The procedure of forming the peptide bond is reported by Maeda and others who is this invention person (Journal of Medicinal Chemistry, 28, 455-461, (1985)).

[0012] the quality of a genuine article and its derivative are local -- passing -- a vein ---like -- it

passes and an artery permucosal target and the endermic and oral thing prescribed a medicine for the patient and injected intraperitoneally are possible.

[0013] That is, it is KTI, for example as a water-soluble medicine for intravenous injections. As it is Or a physiological saline, Use dissolved in glucose solution or other water-soluble injection 5% can be carried out. It can be used also as a tablet RIPIODORU-ized as oils for arterial injections, in addition is KTI. The use which emulsifies dissolved solution using an emulsifier with fats and oils, such as olive oil, medium-chain-fatty-acid ester, linoleic ester, soybean oil, and tung oil, and use with the form of liposome are also possible.

[0014] KTI Or the derivative can be used together with ** which has a the very thing carcinostatic operation. As ** which has a the very thing carcinostatic operation, neo cull chino SUTAN (NCS), SMANCS [what was combined through the amino group of the SMANCS:styrene maleic-acid (SMA) copolymer and neo cull chino SUTAN (NCS)] mitomycin (brand name) etc. -- a carcinostatic antibiotic and 5-Fu (brand name) etc. -- an antimetabolite -- picibanil (brand name) etc. -- an immunostimulator or other carcinostatic medicines can be used -- KTI processing it into one tablet as a mode of concomitant use -- or a single taste object -- each -- the procedure of prescribing for the patient individually is raised.

[0015]

[Function] KTI An inflammatory-edema sthenia inhibitory effect and cancerous ascites, It has a pleural effusion stagnation inhibitory effect, and a survival advantage is shown as a result. KTI The anticancer operation which this ** has is enhanced by using together with ** which has a the very thing carcinostatic operation further. It is in vivo about the Kallikrein bradykinin system considered to involve in the case of the inflammatory edema although the Reason which these effects produce is not completely clear. It sets and is KTI. It prevents effectively. Sthenia of the pain development by the manifestation of the bradykinin and blood vessel permeability is controlled, And the same system as blood-vessel-permeability increase of a cancer organization exists, exsorption of plasma protein of suppression, i.e., this organization, etc. is controlled in blood vessel permeability, and it is pleural effusion, stagnation of the ascites is controlled, And plasma component - feeding in this organization is intercepted, and it is presumed whether to heighten the direct applied force of an anticancer agent. Moreover, the cancer cell proliferation promotion operation by the protease may be inhibited to the part. Furthermore, KTI Since a molecular weight is about 20,000 (a derivative is more than this), there is almost no exsorption to the normal tissue where blood vessel permeability is not accelerating, it contributes to alternative penetration and operation to the organization where blood vessel permeability rose to some extent, and controlling growth of an edema and a cancer organization is also presumed. Moreover, although the decoction (transudate) of an inflammation part is collected from lymph, since there is no systema lymphaticum in tumor tissue, it is thought that the polymer oozed and carried out stops there for a long period of time,

without being collected, and it is thought that this character is also useful to the selective action to the affected part.

[0016] In addition, although obliteration of nutrition supply in a cancer organization is similar with the view of the nutrition supply suppression by physical embolization therapy, compared with a physical embolus having the evil often broken to a normal organization, such side effects are not produced in this invention agent.

[0017]

[Working example] Although the work example of this invention is explained below, illustration is for explanation and is not what meant limitation of invention pneuma.

The soybean whey obtained from a work-example 1 (example of preparation of KTI) low degeneration defatted soybean in process in which separation soybean protein is manufactured is condensed. It is 0.5 to this concentrate (5.5% of crude protein content) 1 **. It is further 1.5 to the supernatant which added acetone of **, agitated for about 1 hour, and was obtained by centrifugal separation. The sedimentation fraction which added acetone of **, agitated for about 1 hour, and was obtained by carrying out centrifugal separation was dialyzed to water. It is 0.5M of 1/50 quantity to this dialysing fluid. - Sodium phosphate buffer solution (pH 7.0) In addition, pH is adjusted to 7.0 and it is DEAE. - It lets it pass to a cellulose ion exchange column. [the eluent which is made to stick to this resin and subsequently has the straight line salt concentration gradient of 0-0.4M] The fraction is carried out with a fraction collector and it is BBI. Type trypsin inhibitor or KTI Salting-out concentration of the rich fraction is carried out further respectively. (BBI CM cellulose ion exchange resin refining type trypsin inhibitor further) Each concentration refining thing is freeze-dried after isoelectric-point sedimentation, and it is KTI. **** and BBI Type trypsin inhibitor was obtained. The purity of each **** is SDS. It is content polyacrylamide gel electrophoresis and is 95% among protein. It was above. Moreover, the former was [1.97Unit /mg protein and the latter of specific activity] 3.37 Unit/mg proteins.

Work example 2 (ascites stagnation suppression and survival advantage)

It is Sarcoma 180 in the abdominal cavity of the ddY-mouse (one groups [seven]) of a female after the birth [the 8th week of]. Soil suspension is transplanted so that it may become 500,000 cels / **. KTI obtained from the day in the above-mentioned example of preparation to one cc of physiological salines Or BBI the water-soluble medicine which dissolved 3mg of type trypsin inhibitor, or an insoluble physiological saline -- one day and every one cc per animal -- four days -- or a medicine was continuously prescribed for the patient into the abdominal cavity on the 14th, and the free athrocytosis and a free water intake were carried out in the meantime. Sarcoma 180 The example which is not transplanted at all non-taken a measure was also carried out in parallel.

[0018] It is Fig. 1 (medication during 14 days) about the result of having performed

measurement of body weight in the meantime. And Fig. 2 (medication during four days) It was shown. By macroscopic observation as a result of being shown in both figures, it is KTI. Inhibitory effect of the weight increase to depend (inhibitory effect of ascites stagnation) It is BBI to having accepted clearly and there having almost been no difference with the group non-taken a measure. Most these inhibitory effects by type trypsin inhibitor were not accepted.

[0019] Furthermore, the average survival time in the above-mentioned example of medication etc. is as in the following table, and the survival advantage was accepted.

[0020]

[Table 1]

投与日数	14日		4日
群	平均生存日数	20日目の生存率（%）	平均生存日数
コントロール	19.1日	28.6	21.4日
KTI	26.0日	100.0	24.8日
BBI型	21.9日	71.4	21.0日

KTI prepared by the procedure of work-example 3 (example of preparation of the derivative combined with the partial butyl-ization SMA) work example 1 [300mg] 30 cc 0.5M It dissolves in sodium hydrogen liquid and is the partial butyl-ization SMA (average molecular weight 2000 [about]). 150mg Glycine solution was added and the reaction was suspended, after agitating for 90 minutes at 25 degrees C in addition. The residual activity of this thing was 36.3%, and the number of the embellished amino group which was measured by the TNBS method (protein, nucleic acid, enzyme, 18 (13), and 1153-1159 (1973)) was 1.94 piece / molecule. Furthermore, it dialyzes and freeze-dries in ammonium-hydrogencarbonate solution, and is KTI. SMA Embellished derivative (KTI-SMA) It obtained.

Work example 4 (inflammatory-edema sthenia restrainer)

Embellished KTI-SMA which was obtained in the work example 3 What was dissolved in the physiological saline is used as a test drug. 1% carrageenin solution which mixed with this test drug or the physiological saline, and was prepared (respectively [the content of KTI-SMA] 5mg [cc] /or 15mg/(cc)) As opposed to SD system male rat (average weight 150g ** 5g, one groups [five]) The result of having investigated the inhibitory effect over a carrageenin induction leg edema was as Fig. 3 by 0.1 cc's carrying out /part medication, causing inflammation, and measuring leg capacity temporally.

[0021] As shown in this figure, the inflammatory leg edema inhibitory effect remarkable in a KTI-SMA 15mg/cc medication group was accepted at the time of 3 hours after inflammation

inducement.

[0022] In addition, KTI-SMA An inhibitory effect is KTI-SMA although KTI 20mg [which is not embellished / which it replaced with and was obtained in the work example 1]/cc was used. It remained in the effect comparable as a 5mg [/cc] medication group.

Work example 5 (effect of the carcinostatic potentiation)

ddY-mouse of a female after the birth [the 8th week of] (one groups [ten]) It is Sarcoma 180 in an abdominal cavity. Soil suspension 50 10,000 It transplants so that it may become cell/**. KTI obtained from the day in said example of preparation to the physiological saline, and neocarzinostatin (NCS) Or repetitive administration of both mixture was carried out once for seven days on the 1st, and the group which prescribed only the physiological saline for the patient was considered as control. The free athrocytosis and the free water intake of during an experimental period were carried out.

[0023] Transition of the dose and the probability of survival (%) was shown in the following table.

[0024]

[Table 2]

群	投与量 mg／kg 体重	生存率(%) 20日	30日	50日
ｺﾝﾄﾛｰﾙ	—	10	0	0
KTI	100 (a)	100	30	0
NCS	0.01 (b)	100	30	0
KTI+NCS	(a)+(b)	100	60	40

It is KTI as the result of an upper table shows. Not only having a survival advantage oneself but carcinostatic (NCS) Having the synergy which heightens an effect was accepted.

[0025]

[Effect of the Invention] It is KTI as explained above. And ** which uses the derivative as an active ingredient has the stagnation inhibitory effect and carcinostatic operation enhancement effect of suppression of the inflammatory edema, and the cancerous pleural effusion ascites. Suppression of the inflammatory edema controls the development or sthenia of a pain by an edema beforehand, and [with stagnation suppression of the pleural effusion ascites] Introduction of a lot of evils by this stagnation, for example, a physical strength fall of a patient, falls of a curative effect, ******, respiratory failure, and quality of the plague etc. is prevented or controlled beforehand, and there is an effect made to live long, such as heightening a curative effect.

[Brief Description of the Drawings]

[Drawing 1] It is the graph which shows the relation between the medication medicine in a work example 2, and weight increase (the amount of ** condensation stagnation).

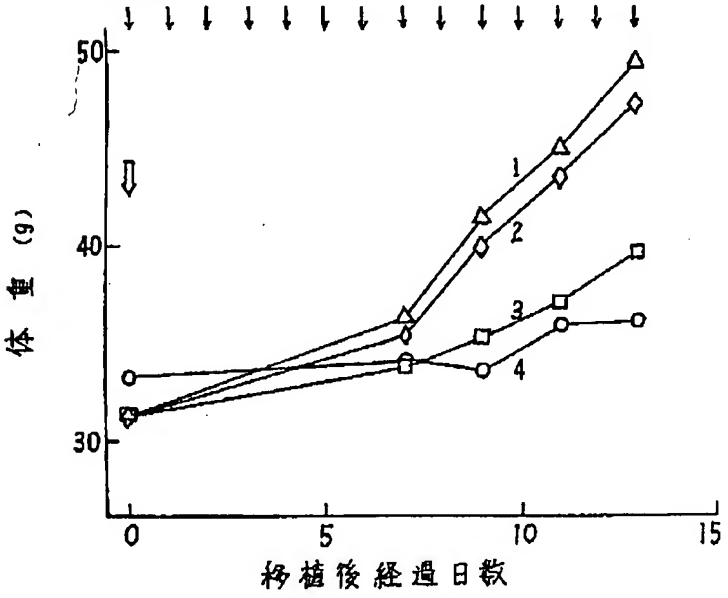
[Drawing 2] It is the graph which shows the relation between the medication medicine in a work example 2, and weight increase (the amount of ** condensation stagnation).

[Drawing 3] It is the graph which shows capacity change of the leg edema in a work example 4.

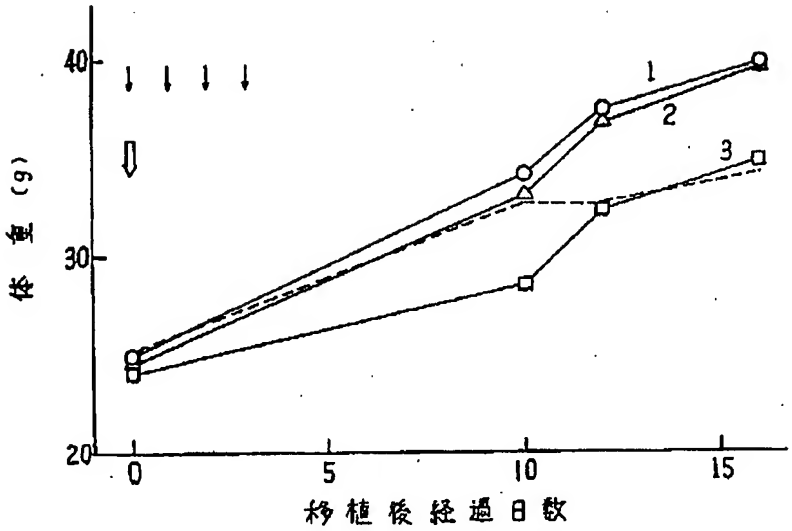
[Explanations of letters or numerals]

Inside of drawing 1 ((1) **) .... A physiological saline medication group (Sarcoma 180 cancer-bearing control), (2), (<>) .... BBI A trypsin inhibitor medication group, (3), (**) .... KTI A medication group, (4), (O) .... Group non-taken a measure (noncancerous control) .... (white arrow) Carcinomatous-implants day (**) .... Medication day. Inside of drawing 2 ((1) O) .... A physiological saline medication group (Sarcoma 180 cancer-bearing control), (2), (**) .... BBI A trypsin inhibitor medication group, (3), (**) .... KTI Medication group .... (chip box dashed line) Group non-taken a measure (noncancerous control) .... (white arrow) Carcinomatous-implants day (**) .... Medication day. Inside of drawing 3 ((1) O) .... A physiological saline medication group, (2), (**) .... A KTI-SMA 5mg /cc medication group, (3), (**) .... KTI-SMA 15mg/cc medication group.

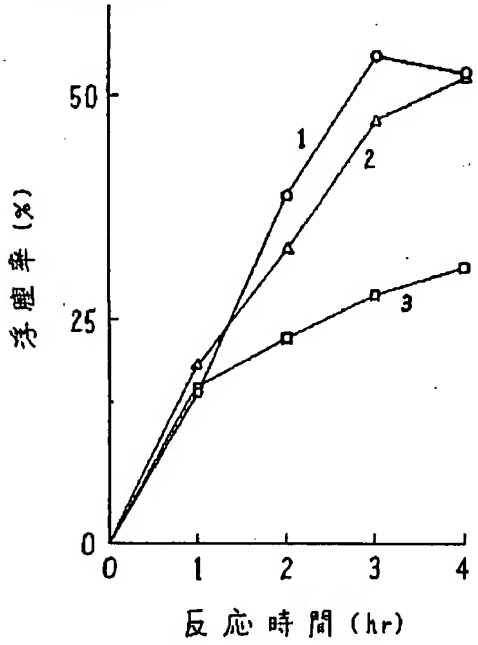
[Drawing 1]



[Drawing 2]



[Drawing 3]



[Translation done.]